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CORRELATIONS BETWEEN THE IN VITRO AND IN VIVO ACTIVITY OF ANTI-HIV AGENTS: IMPLICATIONS FOR FUTURE DRUG DEVELOPMENT

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Some 10 years after the first recognition of acquired immunodeficiency syndrome (AIDS) as a new syndrome, we have identified a number of molecular targets to interrupt the replicative cycle of human immunodeficiency virus (HIV), the causative agent. A number of dideoxynucleosides have been identified as having anti-HIV activity in vitro, and several of these have been found to have clinical activity in patients. In contrast, while a number of agents have been found to block viral binding to the target cell in vitro, these agents have generally not shown clear-cut evidence of clinical activity. Agents which act at a variety of steps in the HIV replicative cycle are now under development, and it is likely that we will have an increased armamentarium to fight this disease in the near future.

KEY WORDS: Anti-HIV agents, CD4, AZT, zidovudinc, ddC, ddl, reverse transcriptase, HIV-protease.

1. INTRODUCTION

The acquired immunodeficiency syndrome (AIDS) epidemic is now in its second decade. Since AIDS was first recognized as a new disease entity in 1981, the causative agent, human immunodeficiency virus (HIV), has been identified and we have accumulated a substantial understanding of the replicative cycle of this agent. HIV is perhaps the most complex retrovirus studied; it thus offers a variety of potential points of attack¹ (Table I). Several antiretroviral drugs have now been developed,² and coupled with better therapy for opportunistic infections, the expected survival of AIDS patients today is consequently longer than it was at the beginning of the epidemic.^{3,4} In addition, the use of anti-retroviral therapy in earlier stages of HIV infection has been shown to delay the progression to frank AIDS, both in controlled randomized clinical trials and in epidemiologic studies.⁵⁻⁸ However, AIDS remains a fatal illness. No curative treatment is available, and there is an urgent need for improved therapeutic strategies. As such, it is perhaps worthwhile to consider the experience to date with certain molecular targets for anti-HIV therapy, to help guide us in developing future approaches.

TABLE I
Steps in the HIV Replicative Cycle which may be Targets for Therapy

Step	Possible intervention
Binding to target cell	CD4 analogues Antibodies to HIV or receptor Non-specific inhibitors (e.g. dextran polysulfate)
Fusion to target cell	Anti-gp41 antibodies Drugs which block fusion
Entry and uncoating of RNA	Drugs to block this step of HIV replication may be found
RNA to DNA transcription by reverse transcriptase	Many active drugs (e.g. AZT and other dideoxynucleosides, phosphonoformate, TIBO derivatives) act at this step
Degradation of RNA by RNase	Specific inhibitors of HIV RNase may be found
Migration to nucleus and integration into host DNA	Agents which inhibit these steps may be found
Transcription and translation	Inhibitors of Tat or Rev activity Anti-sense constructs? (e.g. against tat or rev) TAR decoys (which may bind Tat) Ribozymes may destroy HIV mRNA
Ribosomal frameshifting	Inhibitors of frameshifting may be found
Cleavage of Gag and Pol polyproteins	A number of inhibitors of HIV protease have been developed
Protein modification	Glycosylation inhibitors (e.g. castanospermine) Myristoylation inhibitors may be found
Viral packaging	Antisense constructs against the packaging sequence may be found to have activity
Viral budding	Interferons (may work at other steps as well) Antibodies to viral release antigens Agents which selectively kill cells expressing HIV antigens (e.g. CD4-toxin fusion proteins)

2. REVERSE TRANSCRIPTASE INHIBITORS

2.1. Dideoxynucleosides

Reverse transcriptase, an enzyme encoded by the *pol* gene of HIV, is essential for HIV replication and is for all practical purposes a unique viral enzyme. Soon after HIV was found to be the causative agent for AIDS, several members of the family of compounds known as dideoxynucleosides were found to be potent and selective anti-HIV agents *in vitro*. The development of such drugs depended on the establishment of assays employing live AIDS virus in human cells. A number of these compounds have subsequently been shown to have clinical activity in HIV-infected patients. This work was able to proceed so rapidly in part because of the earlier pioneering work of Horwitz, Ostertag. Furmanski, and others in the 1960's and 1970's. Dideoxynucleosides undergo anabolic phosphorylation in target cells to the active 5'-triphosphate moieties. (The specific metabolic pathways vary from drug to drug.) In a fully phosphorylated form, they inhibit viral polymerase (reverse transcriptase) activity both by acting as chain terminators and as competitive inhibitors of deoxynucleoside-5'-triphosphates. The phosphorylation of dideoxynucleosides to their active moieties is catalyzed by mammalian kinases (HIV is not known to encode for such kinases).

As a result, there are substantial species differences in the anabolic catabolism of dideoxynucleosides, and this is one reason why it is difficult to predict their activity against HIV in human cells from experiments in murine systems. For example, the drug ddC is poorly phosphorylated in murine cells and has little activity against murine retroviruses in such cells; in contrast, it is efficiently phosphorylated in human T cells and is one of the most potent anti-HIV agents in vitro in such cells. ^{10,26} In human cells, different sets of enzymes catalyze the phosphorylation of the various dideoxynucleosides.² Thus, these drugs have different activity and toxicity profiles.

and each has to be considered on its own terms as a separate agent.

At least 7 dideoxynucleosides, including 3'-azido-2',3'-dideoxythymidine (AZT, zidovudine), 2',3'-dideoxycytidine (ddC), and 2',3'-dideoxyinosine (ddI, didanosine), have now been administered to patients, and several of these have been found to have clinical anti-HIV activity. At this point, one can say that within this class of drugs, potent and selective in vivo activity in certain systems using HIV and human target cells can be a fairly good predictor of clinical activity (unless unexpected toxicity prevents administration at adequate doses). Animal toxicity studies have not been reliable predictors of the principal toxicities of this class of drugs. For example, peripheral neuropathy (the dose-limiting toxicity of ddC) and pancreatitis (an important dose-limiting toxicity of ddI) were not observed in preclinical animal toxicity studies (although animal models of neuropathy have subsequently been developed). This again serves as a reminder that the anabolic phosphorylation of dideoxynucleosides varies from species to species, and one must use caution in extrapolating from lower animals to man for the selection of anti-retroviral agents.

The first three dideoxynucleosides to be extensively analyzed in patients were AZT, ddC, and ddI. 11-18 All were found to induce at least transient increases in the number of CD4 cells and decreases in HIV p24 antigen in patients with AIDS or AIDS-related complex. In the case of two of these drugs, AZT and ddI, the drug levels associated with clinical activity were consistent with those that had anti-HIV activity in vitro. 9, 10 In contrast, ddC (Figure 1) appeared to be somewhat more potent in patients than may have been predicted from certain pre-clinical studies. Moreover, this compound appeared to have a relatively greater effect on serum HIV p24 antigen than on the CD4 count. 13, 14 The factors responsible for this quantitatively and qualitatively different pattern of activity are not understood at present. One possibility is that even closely related drugs may exhibit differential effects in various target reservoirs of virus (T cells, monocytes, etc.). As will be discussed below, preliminary results suggest (but do not prove) that combination regimens of AZT and ddC have more activity (and less toxicity) in patients than either drug used alone, and it is possible that this drug will find its greatest usefulness in combination therapy. 29, 30

The potent *in vivo* activity of ddC in patients has spurred interest in related compounds. Two ddC analogues, 2'-3'-dideoxy-3'-thiacytidine (also called 3'-thiacytidine or 3TC)³¹ and 2'- β -fluoro-2',3'-dideoxycytidine (also called 2'-fluoro-2',3'-arabinofuranosyl cytosine or 2'-threo-FddC),^{32,33,34} have both been found to have potent anti-HIV activity *in vitro*, and these compounds are now being studied in Phase I trials (Figure 1). It will be of interest to compare their activity and toxicity profiles to that of ddC. Two other ddC analogues, 2',3'-didehydro-2',3'-dideoxycytidine (also called 2',3'-dideoxycytidinene) and 2',3'- β -epoxy-2',3'-dideoxycytidine, are also active against HIV *in vitro* (Figure 1).^{35,36} By the same token, certain fluoroor amino-substituted analogues of didcoxypurines^{37,38} and lipophilic halogenated

FIGURE 1 The structures of 2'-deoxycytidine (top left) and 5 analogues with potent and selective anti-HIV activity in vitro. Three of these analogues, ddC, the (-) enantiomer of 2',3'-dideoxy-3'-thiacytidine (3TC), and 2'- β -Fluoro-2',3'-dideoxycytidine, are presently undergoing clinical testing in patients with HIV infection. ddC is also being made available to patients in the United States who cannot tolerate AZT or who are failing AZT therapy under the regulatory mechanism of an Open Label Protocol.

analogues of dideoxypurines¹⁹ are active compounds against HIV. These all may be candidates for clinical testing in the future.

The emergence of drug-resistant isolates can confound any therapeutic strategy. In this regard, the clinical response to AZT, the first of these compounds to be tested, was noted to be only transient in some patients; the CD4 count usually rose during the first several weeks, but then fell. It was subsequently found by Larder, Richman, and colleagues that patients on long-term AZT therapy frequently developed resistance to AZT. 40-42 Resistant strains generally had one or more mutations in the HIV pol gene (most frequently $Asp^{67} \rightarrow Asn$, $Lys^{70} \rightarrow Arg$, $Thr^{215} \rightarrow Phe$ or Tyr, or $Lys^{219} \rightarrow Gln$). In general, more than one base pair substitution was needed to yield a resistant phenotype, and this can explain the relatively slow development of resistance to this agent. Interestingly, these resistant strains preserved their sensitivity to

most other dideoxynucleosides (including ddC and ddl), although there was cross-reactive resistance to another 3'-azido-substituted nucleoside, 3'-azido-2',3'-dideoxy-uridine (AzdU). The development of AZT resistance occurs at the time that CD4 counts generally start to decline. However, it has not yet been formally proven that there is a cause-and-effect relationship between these two events, and it is important that researchers continue to study this issue. Studies are now underway to determine how quickly resistance to ddC or ddI develops in a clinical setting. Our laboratory has failed to detect resistance in isolates obtained from patients receiving ddI therapy for up to 2 years. However, two groups recently reported that some HIV isolates obtained from patients receiving ddI for several months had up to 10-fold reduced sensitivity to ddI. Additional studies will be needed to determine the prevalence and clinical significance of ddI (and ddC) resistance. We would expect drug-resistant isolates to be identified in the future in at least a subset of patients receiving long-term therapy with these drugs.

As noted above, dideoxynucleosides act by chain termination of growing strands of DNA. Thus, it should not be surprising that each of these compounds tested to date has had some toxicity. Long-term toxicity is of particular concern in anti-retroviral therapy since this approach most likely requires treatment on a continuous basis, perhaps for the life of the patient. Certain of the toxicities of these drugs appear to be related to their inhibition (as 5'-triphosphates) of mitochondrial DNA polymerase (gamma polymerase). For example, patients on long-term AZT therapy frequently develop myositis associated with mitochondrial dysfunction. 46,47 The pathogenesis of other dideoxynucleoside-induced toxicities is less well understood, and preventing their development would certainly have clinical benefits. As will be discussed below, one approach to this is the use of combination regimens of dideoxynucleosides with different toxicity profiles (for example AZT and ddC or AZT and ddI). At the same time, the problem of toxicity from dideoxynucleosides has spurred interest in finding new classes of anti-HIV agents.

2.2. Non-nucleoside Reverse Transcriptase Inhibitors

During the past several years, several compounds other than nucleoside analogs have been identified as having potent and selective in vitro activity against HIV type 1 (HIV-1) reverse transcriptase. 48.49 The first of these exciting compounds reported were benzodiazepine analogues known as tetrahydro-imidazo[4,5,1,jk][1,4]-benzodiazepin-2(1H)-one and thione (TIBO) derivatives. 48 Several of these derivatives were found to have extremely high therapeutic indices in vitro. Interestingly, the compounds were highly specific for HIV-1; they had little or no activity against a variety of other retroviruses, including HIV-2. Other groups have since reported that certain compounds that shared certain structural features (for example, most have a sevenmember ring with two nitrogen substitutions) had a similar pattern of specificity (including a lack of activity against HIV-2).49 These compounds appear to inhibit. reverse transcriptase by a mechanism that is non-competitive with respect to the primer, the template, the nucleotide, and tRNA. In addition, several members of this group have been found to have very little toxicity when tested in animals. These latter characteristics would appear to be favorable predictors of anti-HIV activity in patients.

Preliminary results from the first of these compounds to enter clinical testing (R 82913 TIBO derivative) indicated that the drug was well tolerated. Serum levels

well above the 50% inhibitory dose (ID_{50}) of 0.0015 μ M were attained in patients. However, while some patients had decreases in HIV p24 antigen, there were no changes in CD4 counts, and overall, there was no clear-cut evidence of anti-HIV activity. The dosing on this trial was limited by drug supply (this particular compound is very difficult to synthesize), and it is still unknown whether any of these drugs will in fact be found to have clinical activity. If they fail to yield clinical benefit as single agents or if the benefits are only transient, it will be of interest to see whether this is because of the development of viral resistance. Second or third generation congeners in this class could address these problems. It is possible that the ultimate role of this class of compounds will be for use together with a dideoxynucleoside such as AZT – combination therapy may slow the development of resistance to both agents.

3. COMPOUNDS THAT INHIBIT VIRAL BINDING

The initial step in the replication of HIV is its binding to the target cell. In most situations, this appears to be mediated by the binding of HIV gp120 Env protein to CD4 on the cell surface. 51.52 It has been found that this step can be inhibited by a

variety of compounds, both in a non-specific and a specific manner.

For example, several polyanionic sulfated polysaccharides (including dextran sulfate and pentosan polysulfate) were found to have anti-HIV activity in vitro. 53-56 However, none of these compounds has been found to have activity when tested in patients with HIV infection. One of these compounds, pentosan polysulfate, was also of potential interest because it was found to inhibit basic fibroblast growth factor (bFGF) in vitro. 57 There is some evidence that bFGF potentiates the growth of Kaposi's sarcoma cells in vitro, 58 and it was hypothesized that bFGF might have some activity against Kaposi's sarcoma. Preliminary results from a trial of pentosan polysulfate conducted at the National Cancer Institute, however, did not reveal evidence of tumor shrinkage, although certain patients may have had a stabilization of their disease (J. Pluda, S. Broder, and R. Yarchoan, unpublished observation).

Why have these compounds not been found to have clinical activity? One possible explanation is that they may fail to adequately penetrate solid lymphoid organs. Also, they may be relatively inefficient at inhibiting cell-to-cell spread of HIV. Another possible reason is that such compounds are highly bound to protein, so and the concentration of free drug in vivo is almost certainly less than that in cultures enriched with 10% fetal calf serum (the usual conditions for in vitro study). Thus, several factors may explain the failure of these compounds to work in the clinic. However, the results with these drugs do serve as a reminder that the identification of activity

of agents in vitro is not a guarantee of clinical activity.

More recently, several laboratories have produced soluble forms of CD4 by recombinant technology (rCD4), and these have been found to inhibit infection by T cells and monocytes by laboratory strains of HIV in vitro at concentrations of 1 to $5 \mu g/ml$. A theoretical advantage of this approach is that rCD4 could prevent the "bystander" killing of HIV-uninfected CD4+ T cells. It has been shown that upon being exposed to free HIV gp120 (as may occur in vivo), such cells may be killed by gp120-specific cytotoxic T cells. By binding to free gp120, rCD4 (or its analogues) might prevent this from occurring.

One question concerning this general approach was whether HIV could under certain circumstances enter cells via mechanisms that do not involve CD4, and if so.

whether this was of physiologic significance. Certain cell lines of neural or muscular origin do not express CD4, yet have been reported to be susceptible to infection by HIV, and infection of these lines is not inhibited by rCD4.⁶⁷ It has been shown that anti-HIV antibodies can enhance the infection of target cells;⁶⁸ 70 under certain circumstances (e.g. in the case of cytomegalovirus-infected fibroblasts), entry appears to occur via Fc receptors, bypassing CD4.⁷¹ Monocytes are important target cells for HIV infection and express Fc receptors, and we wondered if they might be infected by HIV via a CD4-independent mechanism in the presence of enhancing antibodies. However, we did not find this to be the case; even in the presence of such antibodies, infection of peripheral blood monocytes by HIV was completely inhibited by rCD4.⁷² There is some evidence, however, that infection of certain monocytoid lines by HIV may in fact occur via a CD4-independent mechanism in the presence of enhancing antibodies,⁶⁹ and this issue will require further study to sort out.

Another potential problem with the use of rCD4 as anti-HIV therapy was that the serum half-life is rather short (of the order of an hour). To address this problem, chimeric proteins combining the gp120-binding domain of CD4 with the constant part of IgG (rCD4-IgG) have been constructed. rCD4-IgG, which is sometimes referred to as an "immunoadhesin", was found to be similar to rCD4 in its inhibition of HIV infection in vitro. Immunoadhesins were found to remain in the circulation for a longer period of time than rCD4, thus allowing higher levels to be attained. Phase I trials of both rCD4 and rCD4-IgG have shown these compounds to be well tolerated. There was no clear cut

evidence of anti-HIV activity.

One possible explanation for this observation is that the laboratory strains used for the initial testing are more sensitive than the strains existing in patients. Indeed, Daar, Ho, and colleagues have reported this to be the case; primary isolates of HIV were found to be substantially less sensitive to inhibition by rCD4 than HIV-IIIB or LAV (two commonly used laboratory strains). THE Indeed, no substantial toxicity has been observed with rCD4-IgG in patients, and it is possible that doses higher than those employed in the initial trials would be found to have activity. In this regard, preliminary results suggest that relatively high doses of rCD4-IgG may induce increases in the platelet counts in patients with HIV-associated thrombocytopenic purpura. It will be of interest to see if this is the result of an anti-HIV effect.

Ward and colleagues have recently shown that infection of chimpanzees by the IIIB laboratory strain of HIV could be prevented by pre-treatment with rCD-IgG.⁸¹ This is an intriguing result, and it will be of interest to see if similar results in chimpanzees are attained with fresh isolates of HIV. It is conceivable that such agents may eventually find clinical utility in preventing primary infection with HIV or in prevent-

ing perinatal transmission.

4. AGENTS WHICH ACT AT OTHER STAGES OF HIV REPLICATION

4.1. Protease Inhibitors

A number of agents which work at other stages of HIV infection are now under development. As discussed elsewhere in this volume, one of the more exciting classes of anti-HIV agents now under study are the inhibitors of HIV aspartyl protease. The gag and pol proteins are originally translated as a large polyprotein (gag-pol fusion polyprotein) which must then be cleaved by viral protease (a pol gene product) in

order for proper viral function and assembly to occur.82 HIV protease, a 99 aminoacid protein which exists as a dimer, has been purified and its three-dimensional structure determined by X-ray crystallography. This has permitted the rational design of specific inhibitors. 83-87 At least one such inhibitor has now entered clinical trial, and

several are likely to do so in the near future.

Unlike the inhibitors of reverse transcriptase or of viral entry, protease inhibitors act at a late stage of viral replication, i.e. after a DNA provirus has been formed and integrated into the host cell genome. It is thus possible that such inhibitors will have a different profile of activity than the dideoxynucleosides, and clinicians will have to be alert for this possibility. Unlike dideoxynucleosides, protease inhibitors would be expected to reduce the production of infectious virions from cells already infected with HIV. Such an effect may particularly apply to HIV-infected monocyte-derived cells, which can produce HIV for long periods of time without being destroyed by the virus.** In this regard, if any of the protease inhibitors are found to penetrate the central nervous system, it will be of interest to see if they exert a beneficial effect on HIV demential.

4.2. Other Targets

So far, we have focused on three stages in the HIV life-cycle (entry, reverse transcription, and protein modification) for which inhibitors are available. However, as noted above, HIV is one of the most complicated retroviruses studied to date, and agents which act at a wide variety of steps are now in hand or are actively being developed. For example, HIV RNase H has been purified and its 3-dimensional structure determined; this may permit the rational development of specific inhibitors in the near future. Another area of potential interest is the inhibition of HIV integration, which is mediated by integrase, another pol gene product. 90.91 Yet another approach now being investigated is the inhibition of transactivating (Tat) protein function. Tat is a protein containing 86 amino acids which binds to a receptor called trans-acting response (TAR) element on the long-terminal repeat of HIV and promotes efficient production of viral polyproteins. It is essential for efficient HIV replication, 92 and is thus a potential target for anti-HIV therapy. One inhibitor of Tat has now been identified, and this drug has recently entered clinical testing.

A novel approach to the inhibition of Tat is by overexpression of the TAR element.93 Sullenger and co-workers have recently shown that overexpression of TAR-containing sequences ("TAR decoys") in cells using a tRNA transcription unit could inhibit HIV expression in those cells. This general approach (of rendering cells resistant to HIV infection through genetic manipulation) has been termed "intracellular immunization". Gene therapy using this methodology could potentially be used to render cells of the immune system of patients resistant to HIV infection.

Finally we should mention interferon alpha. This compound, which has clinical activity against Kaposi's sarcoma, can partially inhibit HIV replication in vitro.44 Recent studies have suggested that it may also have some activity against HIV in patients, 95 and its use in combination with AZT is now being investigated in several studies.

5. COMBINATION THERAPY

As can be seen, studies of the life cycle of HIV replication has led to the beginning

of rationally-designed therapies, and agents which act at different steps are now under development. The advent of such therapies offers the possibility of developing regimens in which HIV replication is inhibited at multiple steps. Indeed, it is doubtful that any single agent will ultimately be found to be the optimal approach for the treatment of HIV infection.

There are a number of potential benefits of combination regimens. One is the reduction of toxicity. For example, toxicity profiles of the dideoxynucleosides AZT and ddC are markedly dissimilar; the dose-limiting toxicities of AZT are bone marrow suppression and myopathy, while that of ddC is painful peripheral neuropathy. Taking advantage of this, our group at the National Cancer Institute initiated a trial of alternating AZT and ddC therapy. Preliminary results from this trial, which is still ongoing, suggest that some patients can have a sustained anti-HIV effect and that the toxicity from either drug may be reduced as compared to full-dose single agent therapy. Other regimens exploring alternating AZT and ddC are now being tested by the AIDS Clinical Trials Group (ACTG) of the National Institute of Allergy and Infectious Diseases (NIAID). Group (ACTG) of the National Institute of Allergy and Infectious Diseases (NIAID). Also, Pizzo and co-workers have found that an alternating AZT/ddC regimen can provide a sustained anti-HIV effect in children. ddI also has a different toxicity profile to that of AZT, and combination studies of AZT and ddI are now underway in a number of centers.

Another potential benefit of combination therapy may be to prevent or delay the development of resistance. This may apply even to combinations of dideoxynucleosides, as HIV isolates resistant to AZT appear to preserve their sensitivity to other dideoxynucleosides. Indeed, it is possible that certain drugs which rapidly induce resistance if given as a single agent will have clinical utility only when used in combination with other drugs.

Certain combinations of anti-HIV agents appear to have synergistic anti-HIV activity in vitro. 53.56.97-99 and this may provide yet another rationale for combination therapy. One might expect synergistic interactions to occur particularly with agents that act at different steps of the HIV life cycle, and in fact this is one rationale for pursuing combinations of various types. However, certain drug combinations may antagonize each other. For example, ribavirin markedly reduces the anti-HIV activity of AZT in vitro by inhibiting its phosphorylation. This observation serves as a warning against the ad hoc use of drug combinations without adequate in vitro testing.

It is possible that administration of drugs that suppress certain opportunistic infections may indirectly affect the level of HIV replication. Combinations of such drugs with anti-HIV agents may thus be worth exploring. Regulatory proteins produced by certain herpes viruses or adenovirus, for example, can transactivate HIV. 101 and suppression of these viruses might thus indirectly suppress HIV replication. In a similar vein, infection of CD8-bearing T lymphocytes by human herpes virus 6 has been shown to induce expression of CD4 on such cells and render them susceptible to infection by HIV. 102 It is conceivable that inhibition of human herpes virus 6 might thus help in the suppression of HIV suppression.

6. CONCLUSION

As we have seen, anti-HIV therapy has been shown to delay the progression to AIDS and to prolong the survival of patients with HIV infection. However, while we generally can induce increases in CD4 counts with such therapy, such gains are

generally transient with available drugs, and the progressive decline of CD4 counts then resumes. This does not have to be the case; it is quite possible that sustained improvements in the immune function in AIDS will be attainable in the not too distant future. In particular, while combination therapy has not been found formally to be superior to monotherapy in AIDS, it is likely that sustained responses will be most likely attained with a combination of agents.

While restoration of the immune system to its pre-morbid level remains an admirable goal, this may not be absolutely necessary, at least as a short-term goal. Indeed, a retrospective study of patients followed at the National Cancer Institute has shown that fatal opportunistic infections and tumors generally do not occur until the CD4 count falls below 50 cells/mm^{3, 103} Thus, one can hypothesize that maintenance of the CD4 count above that level will yield a marked improvement in survival. It is quite conceivable that through the development of new agents and combination regimens, it will be possible to test this hypothesis in the not too distant future.

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